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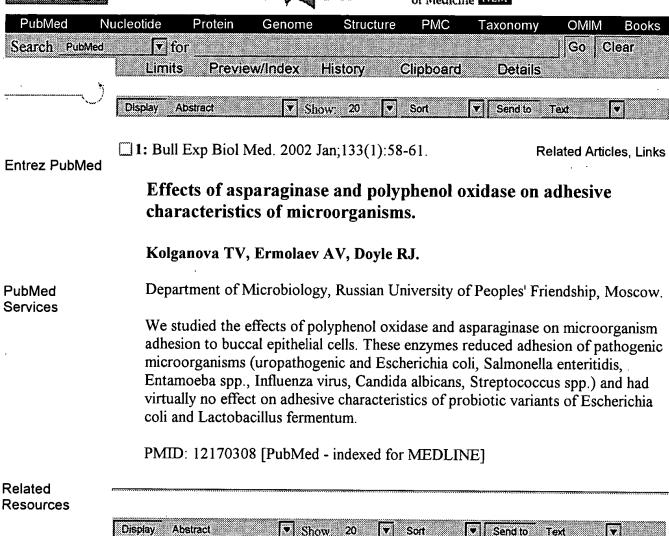


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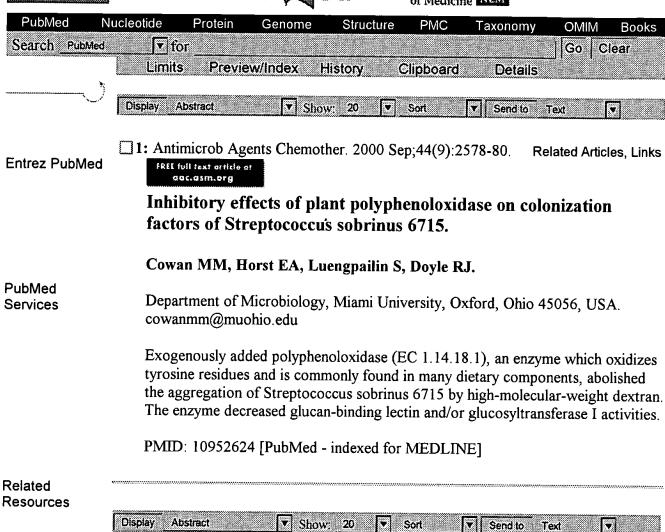












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	Polyphenol oxidase and peroxidase in fruits and vegetables.								
	Vamos-Vigyazo L.								
PubMed Services Related Resources	Polyphenol oxidases and peroxidases are among the and vegetables. Owing to the deleterious effects of differention induced by their actions, these enzymes has of concern to food technologists, while their versatility diversity as protein present a challenge to the biocher account on the present state of knowledge in this field polyphenol oxidases and peroxidases in food and foo and importance in food processing are briefly outlined research including catalytic properties, substrate spectable and temperature, action of inhibitors, isolation, put of the enzymes are given, with special emphasis on reshigh resolution separation and isoenzyme techniques, polyphenol oxidase and peroxidase in selected major vegetables is discussed. Some contradictions found in and some questions that have not been given the necesso far are mentioned.	iscoloration and off-flavor ve not ceased to be a matter ty as catalyst and their mist. This article gives an d. The occurrence of d raw materials, and their role d. Results of biochemical ifficity, susceptibility towards prification, and characteristics excent achievements based on Finally, the behavior of groups of fruits and a the literature are pointed out							
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Partial purification and some properties of polyphenol oxidase extracted from litchi fruit pericarp

Jiang, Y-M., Zauberman, G., Fuchs, Y. (1997) Postharvest Biology and Technology 10:221-228

Litchi (*Litchi chinensis* Sonn.) fruit peel polyphenol oxidase (PPO) was partially purified 21 fold by ammonium sulfate fractionation and gel filtration. Pyrogallol, catechol and 4- methylcatechol were good substrates for the enzyme; with no activity observed with chlorogenic acid, p-cresol, resorcinol, or tyrosine. The optimal pH for PPO activity was 7.0 with 4-methylcatechol, with the enzyme being most stable at pH 7.4. The enzyme was relatively temperature stable with maximum activity at 70°C and requiring a little less than 10 min at 90°C for 50% loss of activity. The Km and Vmax for the enzyme, with 4-methylcatechol, were 10 mM and 1.47x10⁴ units/min per mg protein respectively. The enzyme was not activated by SDS. Reduced glutathione, L-cystein, tropolone, thiourea, FeSO₄, and SnCl₂ markedly inhibited PPO activity, whereas MnSO₄ and CaCl₂ enhanced PPO activity. Data obtained in this study might help to better understand and control commercially, litchi peel browning.

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ACCESSION NO: 9156840

PROJ NO: NYC-149374 AGENCY: CRGO NY.C

PROJ TYPE: CRGO

START: 01 SEP 91 TERM: 31 AUG 95 FY: 1995 GRANT YR: 1993

INVESTIGATOR: Tingey, W. M.; Tingey, W. M.

PERFORMING INSTITUTION:

PLANT BREEDING CORNELL UNIVERSITY ITHACA, NEW YORK 14853

FUNCTION AND EXPRESSION OF POLYPHENOL OXIDASE

CONTRACT/GRANT/AGREEMENT NO: 91-37301-6571

GRANT YEAR: 1993

OBJECTIVES: PROJ. #9101665. Polyphenol oxidases (PPO) are copper metalloproteins which catalyze the oxidation of phenols to quinones at the expense of O(2). The PPO-generated quinones are highly reactive, electrophilic molecules which covalently modify and crosslink a variety of cellular constituents including nucleophiles of proteins such as sulfhydryl, amine, amide, indole and imidazole substitutents. A 59 kD PPOI in glandular trichomes on the leaf surface of wild tomato and potato species appears to be responsible for polymerization of glandular trichome exudate which results in insect entrapment. This PPO is the dominant protein and oxidative enzyme of these organs (ca. 4-80% of total trichome protein).

APPROACH: This proposal seeks to address the expression of 59 kD PPO by identifying cis-regulatory sequences controlling the high-level, epidermis and glandular trichom-specific expression of 59 kD PPO. The DNA encoding the tomato 59 kD trichome polyphenol oxidase gene will be isolated. A series of gene constructs utilizing pBI121 and 5' noncoding regions of the trichome PPO gene will be made to place Beta-glucuronidase under the control of sequences specifying the high-level, epidermal expressions of this gene. Identification of regulatory sequences for the 59 kD PPO may make possible the utilization of glandular trichomes for delivery of bioactive natural products to the leaf surface of crop plants.

PROGRESS: 9501 TO 9512

Polyphenol oxidases convert phenolic substrates to ortho-quinones whose secondary reactions are responsible for much of the oxidative browning evident in fruits and vegetables. However, an in planta function for PPOs has not been determined despite their wide distribution. Roles in plant defense, phenylpropanoid biosynthesis, plastidic oxygen regulation and electron transport have been hypothesized. To gain insight into PPO function(s), we introduced a chimeric antisense PPO cDNA into tomato. Oxidation of caffeic acid, the dominant o-diphenol of tomato foliage, was decreased 39-fold by antisense expression of PPO in transgenic tomato. Immuno-reactive PPO and PPO-specific mRNA and immunoreactive PPO were notdetectable in the transgenic plants. Growth and development were phenotypically normal under greenhouse conditions. However, epidermal leucoplasts displayed an abnormal morphology in antisense plants. PPO-containing protein bodies conspicuous in nontransformed plants were absent or reduced in antisense PPO plants and plastids did not label with antibodies directed

against PPO. However, insect herbivore performance is significantly improved on transgenic tomatoes possessing antisense PPO constructs, indicating that PPO may play a role in plant defense against herbivores.

PUBLICATIONS: 9501 TO 9512

Thipyapong, P., Hunt, M.D., and J.C. Steffens (1995). Systemic wound induction of polyphenol oxidase in potato. Phytochemistry 40:673-676. Hunt, M.D. (1995). Cloning, expression, and functional analysis of polyphenoloxidases. PhD Thesis, Cornell University, 213 pp.

SUBFILE: CRIS

Asparaginase

Scott Hamilton

Function of the Enzyme
Protein Characteristics
Protein Structure
Current Research
References

Function of the Enzyme

The general function of the enzyme Aspariginase is to catalyse the reaction of

L-asparigine + H₂O <---aspariginase ----> L- aspartate + NH₃

The EC number of this particular enzyme is 3.5.1.1 Aspariginase (1)

Protein Characteristics

The enzyme belongs to the protein family: nitrilase, it has a molecular weight of 38,895 Daltons. The amino acid sequence is tggt. (2)

Protein Structure

Current Research

The asparaginase classified as an alpha-be sandwich. The topology a hydrolase. (3)(4)(5)

Insight into the treatment of childhood

<u>(3)</u>

ed by the computer generated images. It is classified as a three-layer alpha-beta-alpha the homologous superfamily is classified as

iduced depletion of antithrombin III in

Asparaginase is the second of the treatment of childhood acute lymphoblastic leukemia (ALL). It appears to work by cleaving asparagine to give aspartic acid and ammonia and thus depleting free asparagine in the blood. Evidence suggests, however, that it can cause a predisposition to thromboembolic problems by significantly decreasing serpin and antithrombin III plasma levels. Human plasma and whole blood showed no loss of antithrombin III activity when treated with theraputic levels of asparaginase. (6)

The L-Asparagine operon of Rhizobium etli contains a gene encoding an atypical asparaginase.

Sequence analysis for the L-asparagine operon of Rhizobium etli was cloned and sequenced. The analysis demonstrated four adjacent open reading frames (ansR, ansP, ansA, and ansB) which coded for a transcriptional repressor, a permease, a thermolabile asparaginase, and an aspartase, respectively. The product of the R etli ansA gene showed a different sequence than previously reported microbial asparaginases, which suggests that it is an atypical asparaginase that may have come form another source other than bacteria or yeast asparaginases (7)

Pegylated asparaginase (Oncaspar) in children with ALL.

Children who would have normally have been treated with native <u>E. coli</u>. asparaginase in induction therapy were switched according to protocol were switched or started on pegylated asparaginase and monitored closely because of observed adverse side-affects of the native asparaginase. Toxicity was low in comparison with the native enzymes, and no allergic reactions were seen. (8)

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1) http://www.expasy.ch.cgi-bin/niceprot.pl?P46011. NiceProt View of SWISS-PROT: P46011

2) http://srs.ebi.ac.uk/srsbin/cgi-bin/wgetz?-e+[BRENDA-ECNumber:'3551']

3) Protein Data Bank, http://www.rcsb.org/pdb/index.html, Structure Explorer http://www.rcsb.org/pdb/cgi/explore.cgi?job=graphics&pdbId=1AGX&page=&pid=123889832

4) CATH (Classification, Architecture, Topology, and Homologous Superfamily of Proteins), http://www.biochem.ucl.ac.uk/bsm/cath_newindex.html, http://www.biochem.ucl.ac.uk/bsm/cath_new/class3/40/50/40/index.html

5) http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?CMD=Text&DB=PubMed

6) Insight into the mechanism of ... http://www.ncbi.nlm.nih.gov/PubMed/. Authors: Bushman, JE, Palmieri, D, Whinna, HC, Church, FC., Department of Chemistry, University of North Carolina at Chapel Hill, USA. Information obtained February 2001.

7) The L-asparagine operon... http://www.ncbi.nlm.nih.gov/PubMed/ Authors: Ortuno, OL, Duran-Vargas, S, Department of Biotechnology, University of Mexico, Mexico. Information obtained February 2001

8) Pegylated asparaginase(Oncaspor) in ... Journal of Haematol, Aug. 2000, pg 379-84. http://www.ncbi.nlm.nih.gov/PubMed/, Authors: Mueller HJ, et al., University of Munster, Germany. Information obtained Feb. 2001

Polyphenol Oxidase

WBC Home
Manual/Protocol Index
Catalog Index

Manual Page | Catalog Page

I.U.B.: 1.14.18.1

Tyrosinase monophenol, dihydroxyphenylalanine: O₂ oxidoreductase

Polyphenol oxidase (tyrosinase) (TY) is a bifunctional, copper-containing oxidase having both catecholase and cresolase activity (Malmström and Rydén 1968):

Jolley et al. (1974) refer to it as an oxygen and 4 electron-transferring phenol oxidase. It is responsible for browning reactions throughout the phylogenetic scale.

Although a tyrosinase from *Neurospora crassa* has been purified (Fling *et al.* 1963), most work has been done with the mushroom enzyme, even though yields and consistency are poor; its multiplicity was shown by Smith and Krueger (1962). Bouchilloux *et al.* (1963) obtained four enzymes. See review by Nelson and Mason (1970).

Characteristics of Polyphenol Oxidase from Mushroom:

Molecular weight: 128,000 (Duckworth and Coleman 1970).

Composition: The enzyme is a tetramer containing four gram atoms of copper per molecule (Jolley et al. 1974), and two binding sites for aromatic compounds including phenolic substrates. There is also a distinctly different binding site for oxygen, the copper site (Duckworth and Coleman 1970). The copper is probably in the cuprous state; inactivation of the enzyme is associated with increase in Cu²⁺. (Kertész et al. 1972). The amino acid composition has been determined. Extensive structural studies have been reported by Jolley et al. (1969); and Duckworth and Coleman (1970). See also Jolley et al. (1972, 1973, and 1974).

Optimum pH: 6.0-7.0.

Extinction coefficient: $E_{\infty}^{1N} = 24.9$ (immediately after purification) (Duckworth and Coleman 1970).

Inhibitors: Compounds that complex with copper. The enzyme is also inhibited competitively by benzoic acid with respect to catechol and by cyanide with respect to oxygen (Duckworth and Coleman 1970).

Activity: Polyphenol oxidase is an oxygen transferring enzyme. Besides using O_2 to catalyze the dehydrogenation of catechols to orthoquinones and the orthohydroxylation of phenols to catechols, a peroxidase activity has been reported on by Strothkamp and Mason (1974). Kinetic studies have been reported by Kertész *et al.* (1971). See also the review by Malmström and Rydén.

Specificity: A large number of parasubstituted catechols areoxidized (Duckworth and Coleman 1970).

Stability: The lyophilized preparation is stable for 6-12 months when stored at 5°C.

Assay

Method: Polyphenol oxidase oxidizes tyrosine to dihydroxyphenylalanine which in turn is oxidized to o-quinone. The latter is accompanied by an increase in absorbance at 280 nm. The rate of increase is proportional to enzyme concentration and linear during a period of 5-10 minutes after an initial lag. One unit causes a change in absorbance at 280 nm of 0.001 per minute at 25°C, pH 6.5 under the specified conditions.

Reagents

- 0.5 M Potassium phosphate buffer, pH 6.5
- 0.001 M L-Tyrosine

Enzyme

Dissolve at a concentration of 1 mg/ml in reagent grade water. Dilute further in reagent grade water to a concentration of 200-400 units/ml.

Procedure

Adjust the spectrophotometer to 280 nm and 25°C. Pipette into each cuvette as follows:

0.5 M Phosphate buffer, pH 6.5	1.0 ml
0.001 M L-Tyrosine	1.0 ml
Reagent grade water	0.9 ml

Oxygenate this reaction mixture by bubbling oxygen into cuvettes through a capillary tube for 4-5 minutes. Transfer cuvettes to the spectrophotometer and record A_{280} for 4-5 minutes to achieve temperature equilibration and to establish blank rate, if any. Add 0.1 ml of appropriately diluted enzyme and record A_{280} for 10-12 minutes. Determine $?A_{280}$ from the linear portion of the curve. A non-linear "lag" of 2-3 minutes can be expected.

Calculation

Units/mg =
$$\frac{\Delta A 280 / \text{m in} \times 1000}{\text{mg enzyme in reaction}}$$

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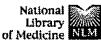
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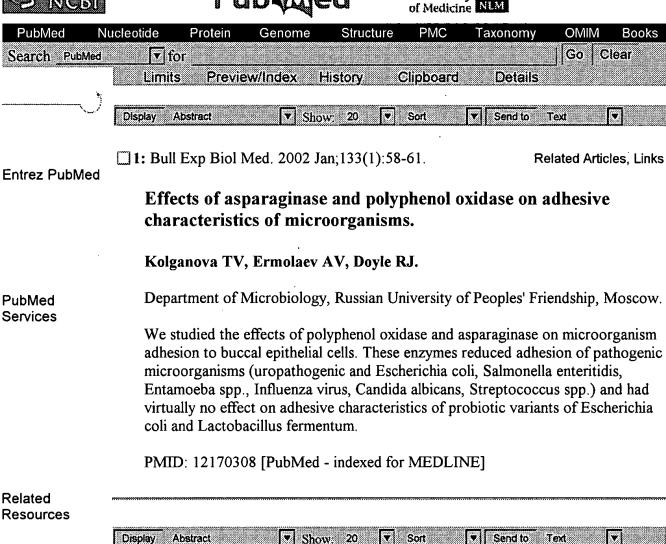
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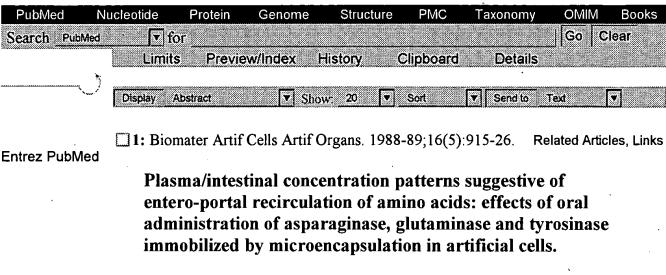
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Chang TM, Lister C.

Artificial Cells and Organs Research Centre, Faculty of Medicine, McGill University, Montreal, P.Q., Canada.

This study suggests the presence of an entero-portal recirculation of amino acids. Endogenous sources of amino acids are secreted at high concentration into the small intestine. Most of the amino acids are absorbed as the content passes down the small intestine. Plasma amino acid concentrations are on the average only 1-5% of the concentrations in the duodunum. This is true even in rats on 24 hours of water and sugar with no exogenous sources of amino acids. For example, the PLASMA:DUODENUM concentrations (mumole/litre) are: Asparagine 37:7164, Tyrosine 94:9579, and glutamine/histidine 409:9708. This entero-portal recirculation of amino acids means the potential of a method for specific depletion of body amino acids by oral ingestion of bioreactants like immobilized enzymes. Preliminary studies used artificial cells to immobilize asparaginase, glutaminase and tyrosinase by microencapsulation. Six hours after 1 oral administration, asparagine, glutamine and tyrosine in the ileum were lowered to 10% of the level of the control. Artificial cells containing no enzymes were used as the control.

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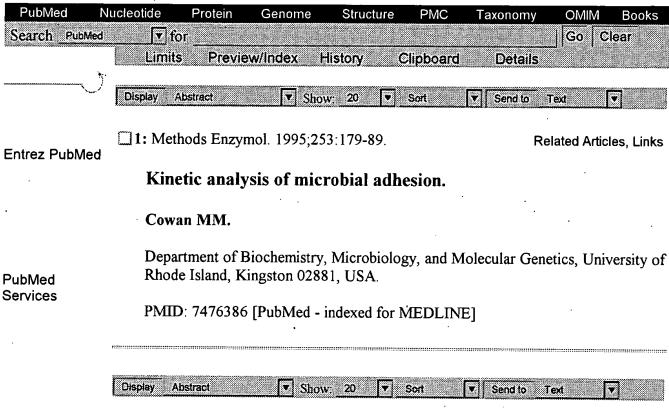
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Response of microbial adhesives and biofilm matrix polymers to chemical treatments as determined by interference reflection microscopy and light section microscopy.

Marshall PA, Loeb GI, Cowan MM, Fletcher M.

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Center of Marine Biotechnology, University of Maryland, Baltimore 21202.

The polymers involved in the adhesion of Pseudomonas fluorescens H2S to solid surfaces were investigated to determine whether differences between cell surface adhesives and biofilm matrix polymers could be detected. Two optical techniques, i.e., interference reflection microscopy (IRM) and light section microscopy (LSM), were used to compare the responses of the two types of polymer to treatment with electrolytes, dimethyl sulfoxide (DMSO), and Tween 20. To evaluate initial adhesive polymers, P. fluorescens H2S cells were allowed to attach to glass cover slip surfaces and were immediately examined with IRM, and their response to chemical solutions was tested. With IRM, changes in cell-substratum separation distance between 0 and ca. 100 nm are detectable as changes in relative light intensity of the image; a contraction of the polymer would be detected as a darkening of the image, whereas expansion would appear as image brightening. To evaluate the intercellular polymer matrix in biofilms, 3-day-old biofilms were exposed to similar solutions, and the resultant change in biofilm thickness was measured with LSM, which measures film thicknesses between 10 and 1,000 microns. The initial adhesive and biofilm polymers were similar in that both appeared to contract when treated with electrolytes and to expand when treated with Tween 20. However, with DMSO treatment, the initial adhesive polymer appeared to contract, whereas there was no change in thickness of the biofilm polymer. These results indicate that both polymers bear acidic groups and thus act electrostatically with cations and are able to enter into hydrophobic interactions.(ABSTRACT TRUNCATED AT 250 WORDS)

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Role of sialic acid in the kinetics of Streptococcus sanguis adhesion to artificial pellicle.

Cowan MM, Taylor KG, Doyle RJ.

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Evaluation of the kinetics of adhesion of Streptococcus sanguis 10556 to saliva-coated hydroxylapatite revealed that sialic acid played a role in the formation of a stable cell-substratum complex. In a previous paper (M. M. Cowan, K. G. Taylor, and R. J. Doyle, J. Dent. Res. 65:1278-1283, 1986) the adhesion was found to take place in two distinct stages: a reversible equilibrium, probably governed by long-range forces, followed by a transition to higher-affinity binding. In the present study, artificial pellicle was treated with neuraminidase, and kinetic adsorption and desorption experiments with S. sanguis were conducted. The depletion of sialic acid from pellicle decreased the initial adsorption rate constant only slightly. The rate constant describing the initial desorption was unaffected. However, no transition to the second (high-affinity) association occurred. While S. sanguis desorption from control pellicles exhibited two sequential rates, with the second rate being approximately 10 times slower than the first, all desorption from sialo-deficient pellicles occurred at one rate that was equivalent to the initial rate constant for control desorption. The cells did not reach an equilibrium with the sialo-deficient pellicle, even after 6 h. Competing sialic acid did not decrease the rate or extent of adsorption, but desorption occurred to a greater extent when cells had adsorbed in the presence of sialic acid. These data suggest that sialic acid plays little role in the initial association of cell and pellicle but that it is necessary for the transition to high-affinity binding and the concomitant decreased propensity to desorb.

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